

The University of Chicago

EFFECT OF ULTRAVIOLET
RADIATION ON GROWTH AND ON
THE CALCIUM AND PHOSPHORUS
CONTENTS OF PLANTS

A PART OF A DISSERTATION SUBMITTED
TO THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1932

By
HARRIS M. BENEDICT

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CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 458

HARRIS M. BENEDICT

(WITH PLATE II AND ONE FIGURE)

Introduction

With the discovery that ultraviolet radiations between 2900 and 3100 Å. caused an increase in the calcium and phosphorus contents of the blood of animals, plant physiologists began investigations to determine whether or not these rays were of importance to plant growth. To date these investigations, which have been so thoroughly reviewed by POPP and BROWN (7) that it is not necessary to discuss the literature here, have not been able to show conclusively that these wave lengths have much effect on the dry weight or on the organic constituents of the plant. With one or two exceptions (BEESKOW 4, and FULLER 10), however, they have neglected the ash elements of the plant.

At the time this investigation was started, no paper had appeared on the effect of ultraviolet radiation between 2900 and 3100 Å. on the calcium and phosphorus contents of plants, and the reported results were so conflicting and open to question that it seemed advisable to investigate this subject further.

For any radiation to affect the object it strikes it must be absorbed by that object; therefore if certain wave lengths are transmitted by a leaf they cannot affect it. If the epidermis of a leaf transmits certain wave lengths which the rest of the leaf absorbs, however, the radiation absorbed will bring about some change in the interior of the leaf. It may be only a rise in temperature, but it may also bring about chemical changes such as in photosynthesis, or it may modify or decompose the constituents of the protoplasm such as enzymes, sugars, amino acids, etc. It is important therefore to study the absorption of the ultraviolet rays by the leaf and to observe any differences that

may occur. In investigations in which glass filters and quartz mercury arcs were used to furnish the different regions of the ultraviolet, the possibility was kept in mind that these glass filters might leak a small amount of the harmful rays shorter than $2900 \text{ \AA}$., which would offset any beneficial effect derived from the region between 2900 and $3100 \text{ \AA}$. On the other hand, in plants which were stimulated under these conditions it was possible that the upper epidermis might filter out these harmful rays, preventing them from reaching the photosynthetic cells directly beneath the epidermis.

From some preliminary experiments to determine the effects of this region of ultraviolet on plant growth, there were indications that after the plants were about four weeks old these radiations had no effect whatever. It seemed possible that this lack of influence might be explained on the grounds that with increasing age the epidermis became more opaque to these wave lengths, finally absorbing all of them, so that they could have no effect on the photosynthetic cells in the interior of the leaf.

As no conclusions as to the capacity of transmission of the leaf epidermis, nor as to the changes in such capacity with age, could be found from previous publications, spectrograms were made of the light transmitted by leaves and their upper epidermises, employing species previously used in ultraviolet experiments.

Methods

Three compartments 12 feet long, 3 feet deep, and 7 feet high were constructed. Each of these was protected from radiations from any other compartment. The only light other than the lights hung in the compartments came in through small windows in the north side of the room, and this extraneous light was kept from the compartments by black curtains hung across the front of them.

In each compartment were hung two 1000 watt tungsten lamps with reflectors and a Cooper-Hewitt Uviol mercury arc equipped with an aluminum reflector. The mercury arcs were suspended between the tungsten lamps and provided the blue and ultraviolet radiations, the tungsten lamps furnishing the longer wave lengths. No attempt was made to filter out the infra-red radiations present.

The ultraviolet radiations were controlled by the filters described

by WITHROW (9). The arc in compartment I was not filtered, but the intensity was reduced to that of the other compartments by the use of three layers of no. 600 Du Pont cellophane. The radiations in this compartment ranged from 2650 to 7200 Å. plus the infra-red rays. The mercury arc in compartment II was filtered with two layers of no. 600 cellophane soaked in a solution of sodium benzoate. This filtered out all the wave lengths shorter than 2900 Å. The mercury arc in compartment III was filtered with two layers of the same

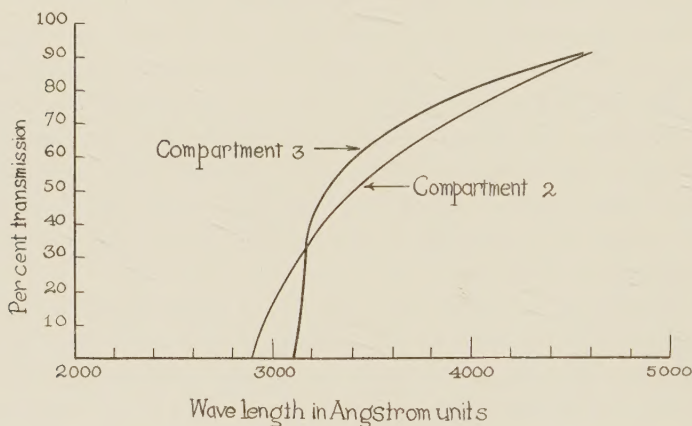


FIG. 1.—Absorption spectra of filters

cellophane soaked in a solution of potassium acid phthalate. This filter absorbed all radiations shorter than 3130 Å.

The absorption curves of these filters are shown in figure 1. As can be seen from the curves, the energy transmitted by the two filters is practically identical, the areas under the two curves being approximately the same. Thus compartments II and III have the same intensity but different wave lengths of ultraviolet radiation. The intensity of the visible radiation in all three compartments was measured with a Macbeth illuminometer and found to be the same, with an intensity equal to 200 foot-candles. The amount of infra-red radiation is not known, but inasmuch as the cellophane filters have little absorption in the infra-red region, it seems safe to assume that the intensity in all the compartments was the same. Recent work by ARTHUR (2) seems to indicate that infra-red rays have little or no

effect on the dry weight of plants. These filters were changed every two weeks and showed no change in transmission in that time. The lights were hung at a distance of 40 inches from the tops of the pots placed in the compartments.

The temperature and humidity of the compartments were not controlled, but records show that the temperatures of the compartments never varied more than 2° C. from one another, with a mean around 25° C. The humidities of the compartments never varied more than 10 per cent, with an average humidity of 50 per cent.

The soil used was a rich loam. It was mixed thoroughly before using to insure equal soil conditions for all the pots in the three compartments. The soil was sterilized and placed in 8 inch pots, forty of which were placed in each compartment. While the plants were growing, the pots were supplied with an equal amount of water once a day.

The seeds were planted and the lights turned on immediately. The lights were in operation from 6:00 P.M. to 8:00 A.M. daily, during which period the curtains were left open in order to keep the temperature as low as possible.

Three series of plants were grown. The first series was planted November 22, 1931 and harvested one month later on December 22. This series will be referred to as series A. The second series was planted January 14, 1932 and harvested two months later, on March 14. This series will be referred to as series C. The third series was planted April 1, 1932 and harvested about six weeks later on May 19. This, which will be referred to as series B, was killed by escaping ammonia gas, but was harvested immediately; it is not felt that for these determinations the results were affected seriously. Each series can be divided into three sets. Set 1 consists of plants grown in compartment I and receiving ultraviolet radiations as short as $2650 \text{ \AA}$.; set 2, of those plants grown in compartment II and receiving wave lengths as short as $2900 \text{ \AA}$.; set 3, of those plants grown in compartment III and receiving wave lengths as short as $3100 \text{ \AA}$. The upper limit of wave length all the sets received was $7200 \text{ \AA}$. plus the infra-red rays. The conditions of the three sets of any one series were as nearly alike as possible, with the exception of the wave length of ultraviolet present.

When ready for harvesting the plants were washed from the pots and the entire plants dried at 100° C. for one hour, then at 60° C. for 12 hours in a ventilated dryer. The plants were then weighed and dry weights recorded.

Calcium was determined by the method involving titration with 0.1 N potassium permanganate (3). Phosphorus was determined by the method outlined by ROE, IRISH, and BOYD (8) for blood phosphorus as modified by COCKEFAIR (5) for use in plant analyses.

The experiments with the penetration of ultraviolet were carried out as follows: A hydrogen arc operating at 4400 volts and one-half ampere was used as the source of radiations. The spectrograms were taken with a Hilger E 3 spectrograph.

The leaves were left on the plant while the spectrograms were being taken. They were placed in front of the slit, held firmly in a flat position, and exposed to the radiation for 20 minutes. This length of exposure was used after it had been found that it did not produce much injury to the leaf, but was of ample length to affect the plate.

In exposing the upper epidermis only, the leaf was placed with this epidermis down on a smooth surface and the lower epidermis and the mesophyll carefully scraped away, leaving only the upper epidermis. This was then examined with a hand lens to be sure that no holes were present. The epidermis was then exposed to the radiation in front of the spectrograph for 30 seconds. It is admitted that this is rather severe treatment, and the epidermis under these conditions may not be normal, but the procedure seems less likely to modify the optical properties than would stripping the epidermis from the leaf.

Results

The results of the determinations of dry weight, calcium, and phosphorus contents of the plants of series A, B, and C are shown in tables I, II, and III respectively. The dry weights are expressed in grams per plant, and the calcium and phosphorus contents as percentage of the dry weight.

In general there is not much difference between the dry weights of the plants of the different sets of series A. In series B and C the dry weights of the plants of set 2 are on the whole larger than those

of sets 1 and 3, with the exception of corn and sunflower in series C where the plants of set 1 have the greatest dry weights.

In all the plants of the three series the calcium contents of those in set 2 are higher than those in set 3, with the plants of set 1 usually

TABLE I

NUMBER OF PLANTS, DRY WEIGHT PER PLANT, CALCIUM AND PHOSPHORUS CONTENTS OF PLANTS OF SERIES A

PLANT	SET 1				SET 2				SET 3			
	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)
Tomato	30	0.011	3.6		31	0.007	2.6		28	0.005	2.1	
Sunflower					40	0.137	3.3	0.62	40	0.205	2.9	0.67
Corn	30	0.173	1.5	0.76	28	0.141	2.0	0.70	23	0.172	1.1	0.63
Soy bean	17	0.161	1.8	0.73	49	0.150	2.4	0.62	43	0.158	1.6	0.67
Lupine	10	0.131	1.7	0.70	29	0.139	1.8	0.77	28	0.141	1.6	0.64

TABLE II

NUMBER OF PLANTS, DRY WEIGHT PER PLANT, CALCIUM AND PHOSPHORUS CONTENTS OF PLANTS OF SERIES B

PLANT	SET 1				SET 2				SET 3			
	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)
Corn	19	0.516	1.9	0.26	16	0.621	1.5	0.68	22	0.515	1.3	0.49
Tomato	30	0.011	3.0	0.35	23	0.185	3.5	0.30	23	0.150	3.2	0.58
Nasturtium	59	0.167	2.4	0.45	50	0.192	2.6	0.47	59	0.166	1.9	0.56
Soy bean	16	0.260	2.0	0.35	28	0.493	2.0	0.54	23	0.460	1.8	0.43
Cucumber	31	0.100	4.4	0.34	21	0.381	4.2	0.54	21	0.258	2.7	0.38

having a calcium percentage intermediate between those of sets 2 and 3.

The percentage of phosphorus is about the same in all three sets of series A. The plants of sets 2 and 3 of series B are about equal in the phosphorus content, which is somewhat higher than that of set 1; while in series C the plants of set 3 have the greatest phosphorus content.

The results of the studies of the penetration of ultraviolet radiations through the epidermal tissues and the entire leaves are shown in tables IV and V. In all cases the transmission of radiation was

TABLE III

NUMBER OF PLANTS, DRY WEIGHT PER PLANT, CALCIUM AND PHOSPHORUS CONTENTS OF PLANTS OF SERIES C

PLANT	SET 1				SET 2				SET 3			
	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)	NO. OF PLANTS	WEIGHT PER PLANT (GM.)	CALCIUM CONTENT (%)	PHOSPHORUS CONTENT (%)
Sunflower....	48	0.230	3.5	0.71	50	0.199	4.0	0.68	32	0.168	3.6	0.68
Cucumber....	20	0.279	3.9	0.45	18	0.441	5.0	0.52	22	0.226	3.4	0.66
Tomato....	21	0.115	3.6	0.65	22	0.263	4.1	0.60	28	0.165	3.1	0.68
Corn....	31	0.875	1.5	0.39	32	0.735	1.5	0.51	31	0.859	1.1	0.58
Soy bean	22	0.304	2.0	0.49	20	0.504	2.2	0.33	19	0.455	0.8	0.54
Nasturtium..	48	0.161	3.1	0.76	38	0.228	2.6	0.54	50	0.137	1.9	0.71

TABLE IV

LOWER LIMITS OF TRANSMISSION (Å.) OF LEAVES AND EPIDERMISES

PLANT	LEAF	EPIDERMIS
Corn.....	3750	2350
Morning glory.....	3420	2250
Cucumber.....	3250	2400
Tomato.....	3850	2240
Wax bean.....	3850	2250
Bryophyllum.....		2500
Radish.....	3500	2300
Coleus.....	3800	2600
Sunflower.....	3550	2260
Soy bean.....	3500	2500
Sudan grass.....	3450	2260

continuous down to a certain wave length, with no radiation shorter than that wave length transmitted. Table IV shows in the second column the shortest wave length transmitted by the entire leaf and in the third column the shortest wave length transmitted by the upper epidermises of the leaves.

Table V shows the transmission of leaves and their upper epider-

mises at the ages of two, three, and five weeks. The results show a slight decrease in the transmission of both the leaf and the upper epidermis with increase in age. In no case does the upper epidermis filter out all the wave lengths shorter than 2900 Å. Plate II shows a typical spectrogram of the transmission of various leaves and their epidermises.

TABLE V
TRANSMISSION (A.) OF LEAF AND EPIDERMIS OF VARIOUS
PLANTS AT DIFFERENT AGES

PLANT	LEAF			EPIDERMIS		
	AGE IN WEEKS			AGE IN WEEKS		
	2	3	5	2	3	5
Wax bean.....	3250	3450	3500	2300		2310
Corn.....	3520	3740	4050	2250	2400	2380
Lupine.....	4800		4800	2270		2300
Radish.....	3330	3340	3800	2240	2240	2300
Sunflower.....	2900		3550	2230		2200
Cucumber.....	3150		3250	2360		2460
Tomato.....	3800	3850	3950	2220	2240	2240

Discussion

The chief criticism of the conditions under which the plants in these experiments were grown was the low light intensity. For good growth the intensity in the compartments should have been around 1000 foot-candles instead of 200. Since the intensity in all the compartments was the same, however, the results can be considered as indications of the effect of the wave lengths between 2900 and 3100 Å. on the growth of the plants studied; but because of the low light intensity they can be considered as indications only.

ARTHUR (1) has recently stated that the potassium acid phthalate filter transmits certain wave lengths shorter than 3100 Å. This is true if the filter is not used with a Corex D or Uviol glass backing. If this filter is backed with one of these glasses, however, as it was in these experiments, there is no transmission of wave lengths shorter than 3100 Å.

The results of the dry weight determinations on the plants of series A indicate that any effect the ultraviolet regions between 2900

and 3100 Å. and between 2650 and 2900 Å. might have on the growth of the plants does not become apparent until after the plants are a month old, since the plants of the different sets of this series showed little differences between their dry weights. The plants of sets 1 of series B and C, which were six weeks and two months old respectively, showed the harmful effects of the rays between 2650 and 2900 Å. in their decreased dry weight and in the burned appearance of the leaves which were exposed to these radiations. The plants of these two series also showed the possible benefit of the wave lengths between 2900 and 3100 Å. in the increased dry weight of the plants of set 2 which were exposed to these radiations as compared with that of the plants of set 3 which did not receive these wave lengths. This increase in dry weight was most marked in the case of tomatoes and cucumbers, being as much as 90 per cent in the cucumber plants of series C. In both series B and C there was enough difference between the plants of the different sets to tell them apart readily. The plants of set 2 were the largest, with the exception of corn in series C; those of set 3 were somewhat smaller; those of set 1 were usually of a stunted appearance with their leaves inrolled and looking burnt.

From the results obtained under the conditions of these experiments, there are indications that in general wave lengths between 2900 and 3100 Å. stimulate plant growth, and that wave lengths shorter than 2900 Å. are harmful to plant growth.

The calcium content of the plants exposed to the wave lengths between 2900 and 3100 Å. was increased, both in percentage and in total amount per plant. All the plants of sets 1 and 2 in all three series showed this increase in percentage of calcium. The plants of set 2 usually had a slightly higher percentage than those of set 1. These results seem to indicate that the wave lengths between 2900 and 3100 Å. are most effective in bringing about this increase in calcium content, and that the wave lengths shorter than 2900 Å. exert very little influence, possibly even interfering with the intake of this element, since the percentage of calcium in the plants of set 1 was generally not so great as in the plants of set 2.

This increase in calcium started with the early development of the plants. The plants of series A were only a month old and showed no significant differences in their dry weights, yet the increase in the

percentage of calcium in the plants of sets 1 and 2 was already apparent. There are no indications in the results that the percentage of calcium increases or decreases with age. For a given series the soil of the sets was identical (that is, all the plants of one series were grown in the same soil), but the soil used in series B was not the same as the soil used in series A, and the soil used in series C was not the same as was used in series B. This difference between the soils of the different series may account for the differences noted between the plants of the three series.

The results of the calcium determinations seem to indicate that wave lengths between 2900 and 3100 Å. bring about an increase in the calcium content of the plants, and that wave lengths shorter than 2900 Å. have little effect on the intake of this element.

The results of the phosphorus determinations are peculiar. The plants of set 1 of series A show an increase in phosphorus over the plants of set 3, but in the other two series this set shows on the whole a decrease in percentage from that of set 3. The plants of set 2 show an increase in phosphorus in series A and B, but a rather decided decrease in series C, sunflower and nasturtium being exceptions. These results are so variable, however, that it does not seem safe to make deductions as to the effect of these regions of wave lengths on the phosphorus content of the plants studied.

The results of the study of the transmission of the leaf and upper epidermis with increase in age do show a possible increased absorption of radiations as the age increases, both in the leaf and in the epidermis. In most cases the variations do not exceed the limits of variation between leaves of the same age, with the possible exception of corn. Likewise the increase in absorption of ultraviolet rays by the epidermis with increase in age does not become great enough to filter out all the radiations shorter than 3100 Å.

The results of the penetration studies show that in no case did any wave length shorter than 2900 Å. penetrate through the leaf, and in no case did the upper epidermis filter out all the wave lengths shorter than 2900 Å. ELTINGE (6) reported that in the case of tomato, cucumber, radish, and *Coleus* she obtained stimulation by adding the wave lengths between 2900 and 3100 Å., but no stimulation in the case of morning glory, wax bean, and sunflower. The results presented here show that the epidermis could not have acted

as a protection against short wave lengths leaking through faulty filters in the case of the stimulated plants, and at the same time have failed to act as a protection to the plants that were not stimulated, since the transmission of the epidermis of all the plants was about the same.

The evidence presented by these tests indicates that any results obtained with ultraviolet radiation experiments must always be interpreted on the basis of the penetration of rays shorter than $2900 \text{ \AA}$. through the upper epidermis of the leaf, and the absorption of these rays by the underlying tissues if they are present in the radiations.

It will be noted that some leaves absorb more ultraviolet radiation than others. This is in general related to the thickness of the leaves. The thick leaf of *Bryophyllum* transmitted no radiations at all; lupine with a relatively thick leaf absorbed all the ultraviolet, and wax bean with a leaf thinner than the lupine leaf absorbed most of the ultraviolet radiations.

Summary

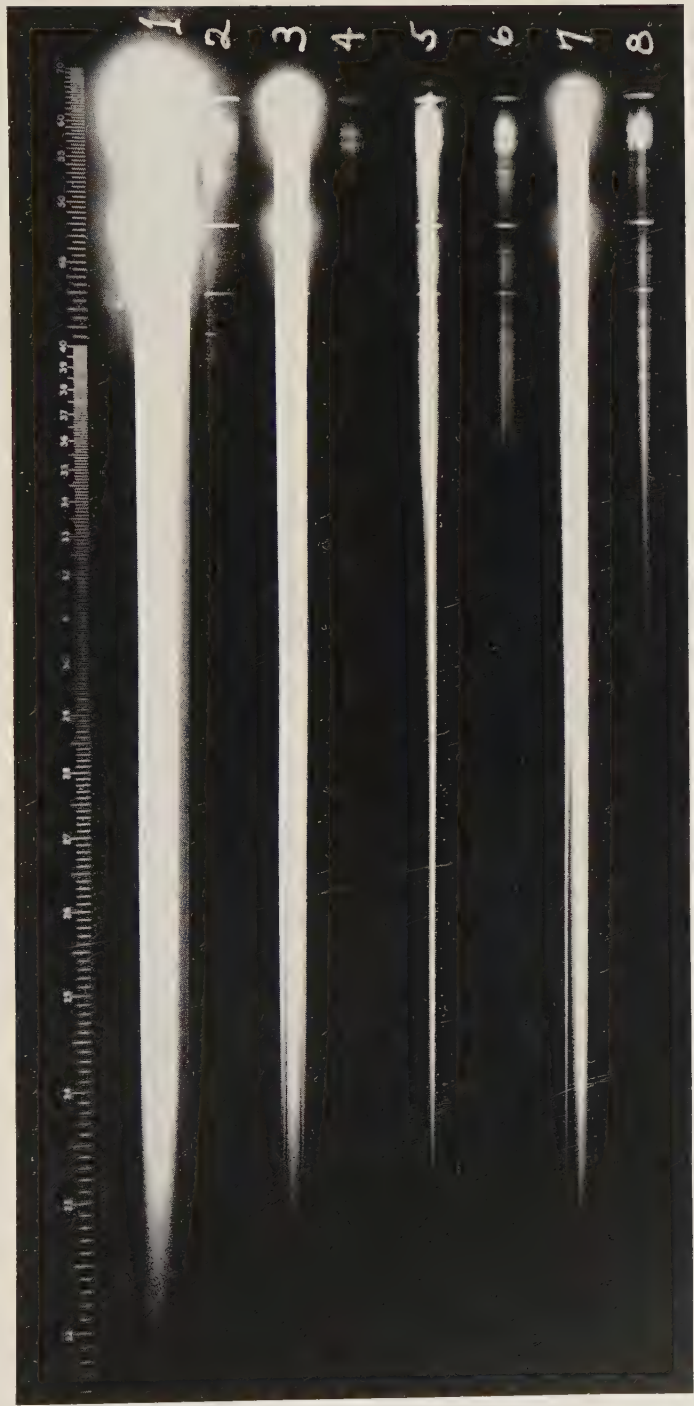
1. Three series of plants were grown and each series was divided into three sets. All the conditions of the three sets of one series were identical with the exception of the range of wave length of ultraviolet radiation received.

2. The amount of growth of the plants was determined by measuring the dry weight. The calcium and phosphorus intakes were also determined for these plants grown in the three different ranges of wave length.

3. The region of ultraviolet between 2900 and $3100 \text{ \AA}$. apparently caused, under the conditions of these experiments, an increase in the dry weight of all the plants grown with the exception of corn. Plants receiving no wave lengths shorter than $3100 \text{ \AA}$. were considered as controls.

4. This same region of ultraviolet (2900 – $3100 \text{ \AA}$.) apparently caused an increase in percentage of calcium in all of the plants grown. No conclusions could be drawn from the results as to the effect of these wave lengths on the phosphorus content.

5. The presence of wave lengths shorter than $2900 \text{ \AA}$. caused a decreased dry weight of the plants but had little effect on the calcium content.



BENEDICT on ULTRAVIOLET RADIATION

Spectrograms of various leaves and their epidermises. Reading from top to bottom: 1, hydrogen arc; 2, tomato leaf; 3, tomato epidermis; 4, lupine leaf; 5, lupine epidermis; 6, radish leaf; 7, radish epidermis; 8, soy bean epidermis. Some faint lines showing the lowest limits of transmission are lost in reproduction.

6. Transmission studies with spectrograms indicate that the upper epidermis cannot filter out harmful wave lengths of ultraviolet, and that its transmission of ultraviolet radiations does not decrease with age.

The writer wishes to acknowledge his indebtedness to the General Electric Vapor Lamp Company for the loan of the mercury arcs used in these experiments; to the Department of Physics of the University of Chicago and to Dr. H. B. LEMON for the use of the spectrograph. He is also indebted to Dr. C. A. SHULL for advice and criticism throughout the progress of this work.

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